BLACK FLIES (DIPTERA, SIMULIIDAE) AS POSSIBLE
VECTORS OF LEGWORM (ONCHOCERCA CERVIPEDIS) IN
MOOSE OF CENTRAL ALBERTA

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Abstract: A filarioid nematode of moose (Alces alces) known as "legworm" and its possible vector, the black fly (Simuliidae), were studied in Swan Hills, Alberta from 1975 to 1977. Legworms recovered from the subcutaneous connective tissue of moose were identified as Onchocerca cervipedis Wehr and Dikmans, 1935. Sixty-four percent of the moose examined were infected with adult legworm. Most adult worms (801) were in the forelimbs. There was a linear increase in number of adult legworms with increasing age of moose. Onchocerca cervipedis microfilariae were present in the skin of the fore and hind limbs of moose during June and July only.

Fifteen species of black fly were collected from live-trapped and a tame, penned moose. Individuals of Simulium decorum, S. venustum, S. vittatum, S. arcticum, S. aureum, and Prosimulium formasum took blood reals. Microfilariae of O. cervipedia were found only in blood meals of S. decorum and S. venustum, incriminating these species as possible vectors of moose legworm.

Rec'd: July 27/81
Order No.
Price: 61+1
Acc. No. 10.11 Crange
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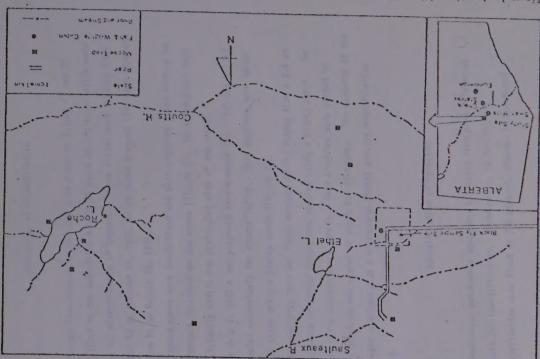


1935 (Hemitoda, Onchocercidae), a parasite of North American cervids, 1) confirm the identification of O. corripadio in moose of Alberta; 2) determine the objective of the study was to provide a better understanding of this 4) determine the identity of adult black files blood-feeding on moose The filarioid nematode checkeres cervipedia Hehr and Dikmans, prevalence of legworm in relation to host age; 3) determine temporal was studied in moose (Alcoo alcoo) from Swan Hills, Alberta during Black files feeding on moose were studied as well. and spatial distribution of microfilariae of legworm in moose; in Swan Hills; and 5) identify possible black fly vector(s) of to: Specific goals were host-vector-parasite system. O. osrvipadia. 1975-1977.

during the study, but due to limitations on time and since a black fly, black-talled deer (Odocoiteus homionus columbianus) (Welnmann et al. Many species of hematophagous files were collected from moose Prosimulium impostor, is a vector of O. carvipadis in Columbian 1973), only black files were examined in detail.

### THE STUDY AREA

zone between the Transcontinental Boreal Forest and the Rocky Mountain 'lodgepole pine'(Pinue contorta), Englemann spruce (Picea angelmanni), The area is a transition characteristic species from both biomes, with species such as The study site was located on the eastern fringe of the The flora is comprised of Swan Hills, in Central Alberta (Figure 1). Subalpine Forest (Cordilleran).



CONTRACTOR R.

and subalpine fir (Abies Lasiocarpa), of the Cordilleran region and white spruce (Picea glawca), black spruce (Picea mariana), and Jack pine (Pinue banksiana) of the Boreal Forest (Anonymous, 1976).

The climate is continental and characterized by cold winters and short, cool summers. The average January temperature is -15°C and the average July temperature is 15.6°C. The average May-September precipitation is 51 cm (Langley 1967).

### METHODS Collection of Adult Legworms

In autumn 1975 a roadside check station (Figure 1) was established on the main highway into the study area in co-operation with Alberta Fish and Wildlife Division. Hunters were asked to submit all four legs cut just below the tibio-tarsal joint, and the head and hide of moose to the check station. Parts of 43 moose were submitted by hunters; rarely were submissions complete (n=1). All (n=36) or some of the legs (n=6), lower jaw (n=17), head (n=12) and hide (n=1) were submitted. Skins of two moose were provided by the Alberta Fish and Wildlife Division.

The tendons of the legs were cut and removed from the tarsal bones to expose underlying connective tissue. These freshly-skinned areas were examined for adult 0. cervipedis. Worm location was mapped and sex and condition of worm noted. Worms were removed from the connective tissue using needle-point forceps, dissecting needle and scalpel, and stored in 70% ethans?

## Collection of Microfilariae

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Sixteen live-trapped modse (see trap sites on Figure 1) and 21 moose killed in the Swan, Hills within 24 hours before examination were sampled for microfilariae of legworm. Moose killed were sampled from September 12 to October 12, 1975, while moose trapped live were sampled from June 15 to August 18, 1976.

Trapped moose were immobilized and skin biops'es taken from up to nine standard locations on the moose (Figure 2). No attempt was made to consistently sample one particular side of the moose.

On July 2, 1977 a two-year-old female moose (No. 122) was killed and sampled extensively for microfilariae. Skin sections were taken from 135 sites using a biopsy punch (Figure 3).

The overlying hair was first removed during blopsy than a 0.4 cm diameter core of dermal tissue was removed using a blopsy punch. Two adjacent cores constituted a sample. Skin blopsies were placed in Earle's Solution and stored in vials for 24 hours. The contents of the vials were examined with a microscope (100X) and the microfilariae counted.

## identification of Parasite

Onchocerca cerripedie specimens collected from nine wild moose were used for identification. Seven males, 10 females and 16 microfilariae were fixed in 70% ethanol, cleared in beechwood

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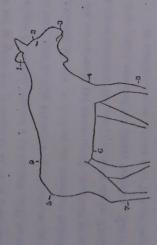


Figure 2. Blopsy sites for collection of microfilariae.

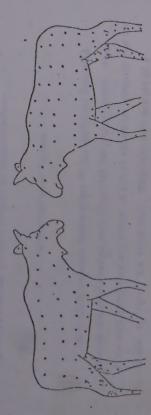


Figure 3. Sites of biopsies for collection of microfilariae from Moose 122 with location and numbers of microfilariae recovered.

creosote and lactophenol (1:1) and examined using a compound microscope. Measurements of all characters were made with a calibrated ocular graticule, except for the total length of female specimens which was determined using a metric ruler. Specimens were identified following descriptions of Wehr and Dikmans (1935), Annereaux (1941) and Caballero (1945) for adult 0, carripadia and that of Hibler (1965) for microfilariae.

Black Fly Adults Attracted To and Blood-Freeding on Moose

Black files attracted to and blood-feeding on moose were collected from inmobilized wild moose (n=13) and a penned moose used as beit.

Moose were swept for black files using an insect net. Individual engorging files were picked from immobilized moose with forceps and a paint brush moistened in alcohol and stored in 70% ethanol.

An orphaned, hand-reared, female, moose calf (No. 3) (approximately six weeks old) was taken to the study site on May 16, 1976. This roose was used to facilitate regular sampling of black files were collected and feeding on it during 1976 and 1977. Black files were collected from it using a sweep net and a fly trap baited with the moose. A sweep net sample consisted of 20 sweeps over and eround the length of the standing moose. A regular sampling schedule was maintained on alternate days at 0900, 1700 and 2000 hr. from May to August in 1976. In 1977, samples were taken every third day at 0900, 1700, and 2000 hr.

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The moose-baited fly trap was a rectangular enclosure constructed of plywood and insect screening (Mudson 1977). A one-way fly entrance ran the length of the trap on either side. The trap was cleared of flies immediately prior to introducing the moose to the trap. A sample consisted of flies trapped in a one-hr period. A regular sampling schedule was maintained on alternate days at 0900, 1700 and 2000 hr. An unpublished taxonomic key by F. J. H. Fredeen (Simulifidae Check List of Species in Manitoba. Saskatchewan and Alberra) was the source for identification of adult black flies, but published works by Stone and Jamnback (1955). Abdelour (1968) and Peterson (1970) were also used. Selected specimens of each species were submitted to Or. Research Branch, Biosystematics Research Institute, Agriculture Canada for confirmation or identification.

## Determination of Vectors

trapped and penned moose, were dissected for microfilariae. Glack files were identified and placed in hot 2% potassium hydroxide for 10-15 min. to liquify the blood meal. They were then mounted on a microscope slide, and the abdomen removed. The intact blood meal was removed by applying gentle pressure with forceps to the abdomen behind the meal and moving the forceps atteriorly. A cover slip was placed over the blood meal and squeezed to form a thin-layer blood squash. This was examined for microfilariae under a compound microscope (100x).

### RESULTS Adult Legmorm

The 45 moose examined for adult 0. corresponds consisted of three complete moose, 42 sets of legs (36 - all 4 legs, 5 - 2 legs, 1 - one leg), 11 heads (18 lower jaws for aging) and one hide.

Adult 0. cervejadia occurred in 29 (641) moose. All worms were located in the subcutancous connective tissue beneath the skin of the legs, except for two located in the brisket and belly area of a heavily infected moose. Eighty percent (475) of the acult worms recovered were in the lower forelegs while 20% (118) were in the lower hind legs. In the leg, 38% (223) of the legworms were in the tiblo-tarral joint area, 56% (334) in the tarsus and 6% (36) in the phalanges.

The moose calf and two yearlings were not 'nfected; most (15) of the 18 adult moose of known age were infected. There was a positive linear relationship between the number of adult legworn per moose and age of moose (correlation coefficient r-0.75, N-21, P-0.05).

Only 13 of 595 adult O. cervipadia recovered in this study were males (2.2 males:100 females). Adult legworms were either in an extended or coiled position. Coiled worms were found either loose in the subcutaneous connective tissue or surrounded by fibrous tissue. Leyworms were either normal or "calcified" in appearance. Calcification was apparently not influenced by host age (correlation coefficient r-2.13 N\*12, P>0.05), or abundance of O. cervipedie (correlation coefficient r-2.13 n\*12, P>0.05), to the host. Neither was it related to the

coiled or extended state of the parasite ( $x^2$ -3.45, p>0.05). Nodules contained from one to seven legworns; most (71%) had only one female with microfilariae in the uterf. Only one male legworm was recovered from a nodule, with one female also-with microfilariae in the uterf.

### Microfilantae

Skin samples were taken from 21 moose killed by hunters in September-October 1975 and 16 moose captured live between June 15 and August 18, 1976. Microfilariae were recovered from eight of the live-trapped moose (Table 1), but not from moose killed by hunters even though 8 of these moose had adult legworms and microfilariae survived in moose skin for at least 48 hrs.

The number of microfilariae recovered varied substantially among individual moose and biopsy sites (Table 1). Microfilariae were found only in skin of the bind legs of 5 moose, only in the front legs of 1 moose and in the front and bind legs of 2 moose.

Afcrofilariae were found only in the skin of the hind legs of the two-year-old moose (No. 122) sampled extensively in July 1977 (Figure 3). Vicrofilariae were recovered from demail tissue samples taken from the medial aspect of the upper and lower legs (n-82) and the lateral aspect of the upper hind legs (n-30).

Table 1. Aumber of microfilarise of Omehroened curvipedie in skin biopsies from recese during 1976.

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## Identification of Parasite

Description of Adult

Adults show characteristics of numbers of the genus Ombhosenen. The coult is fillform and typered at soth ends with fine transverse striations on the cuticle, a simple oral opening, long and narrow esophagus and an intestine only slightly wider than the esophagus.

\*Assumements of disynastic characters are given for female and male worms (Table 2 and 3). The long spicule of the male has heavily chiffinized walls with a disponal continuized ridge in the middle. In lateral view, it is tapered distably to a fine curved point. The short spicule also has thick criticitized addess. The anterior extremity is concave with heavy chitfinized edges in lateral view. It tapers classily and considers as spatched enough rounded edges. Perfanal Tapiliae here about readily scene in collique, lateral-ventral view; two points of addess' papillae along the vertral midline, four pair of publice in close grouping lateral to and one pair posterior to the anus, and one large pair of papillae postitioned near the tip of the land.

# Description of Microfilariae (Table 4)

Victofflantum, like the adults, and filiform and transversely started. They are rounded unteriorly and tapered posteriorly. No sheath was apparent.

Table ?. Messurements of some important features of fenals chackage corpsipedia.

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Table 4. Measurements of some diagnostic characteristics of

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2		Range	224-322	5.8-7.3		Activi		the per	d condi	to blac	of all	, 1976,	is than	e body	ere ha	
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This study

used ear twitching, head and body shaking, and scratching in an appart



effort to disladge files. On occasion, it reced erratically around the pen for long periods, apparently in response to files, then would lie down. Most commonly, it would lie under low shrubs or in a lean-to-shelter constructed to offer some protection from adverse weather.

Wild, trapped moose and the penned yearling moose moved around constantly during peak activity of biting flies, frequently twitching the ears and rubbing the hind legs together in a quick, jerking motion. Ruscle spasms, in the form of quivering along the body core and the legs, were also observed.

## Feeding Sites of Black Files

Oncervations clearly thowed that block files concentrated their probing and feeding activities in the less dense and short-haired areas on the moose. Most activity occurred on the legs, in particular the inner and outer aspect, from the hoof to about 10 cm above the tibio-tarsal joint. Other preferred sites included the belly, brisket, and anal areas. Few black files were observed on the head and ears. A hairless callused area on the hind legs just distal to the tibio-tarsal joints, a preferred site for the moose fly byperosiops sp., [possibly L. alcia (Muscidae, Ofptera)], was used occasionally by adult black files.

## Black Files Attracted to Moose

Fifteen species of black flies were attracted to moose (Table 5). In 1976, when adults of 11 black fly species were collected from the

Table 5. Black filtes attracted to moose in the Swam Hills

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	1075	1076	10101			of management
Species	Sweep	Hand	Sweep Ryc Tria	True	The state of the s	
Similion arotionom	+	+	to comments of the comments of	1	+	4
S. decorum .	+	+	÷	+	*	•
S. venuution	+	+	÷	÷	+	
S. vittation	+	+	÷	÷		
S. meridionale	+	,	à	4		. 4
S. furculatum	ż	1	٠	÷		
S. euryadminiculum	,			•	•	
S. pugotensa	,		,	÷	,	. ,
S. aureum		,	,	9	*	- 4
S. Latipos		,	,	å		٠
S. aroxtoni	1	\$		a		• •
S. Jamingor	+	3	1	8	1	
Provinutium formovum	,	,	+	+	*	٠
P. decumantioulation	1	à	1	4	+	
P. crigenu	1	1	,	+	2	÷



methods showed similar trends in black fly abundance, however, 6 species the most abundant (Figure 4). Of these species, adults of S. ducorum S. Vermaseum, S. ameticum, S. vittabum, and Prosintitum jornesum Were insect sweep net (Table 5). All but S. maridionale were taken in low of black flies were taken in the moose-baited trap and not in the numbers. The disturbance created while "sweep netting" around the moose and the short sampling period may have adversely influenced moose-baited fly trap and insect sweep net, Strutium decorum, and S. venuation were must abundant from late June to August. trapping some of these less appndant black fly species.

In 1977, the numbers of black fly adults attracted to moose were The summer of 1976 was unusually wet and was followed by a mild winter with above higher (Figures 5, 6 and 7) than in 1976 (Figure 4). somal snowfall.

(Figures 5, 6 and 7). Of these, S. cheenum, S. venustum, S. areticum In 1977, when the penned moose was a yearling, 13 species of 5. venuation, 5. aroticism, 5. vittatism, 5. awaim, and P. formosum plack fly were collected; the most abundant were: S. docomum, and S. vittation were abundant in June and July.

Adults of six black fly species were collected from immobilized, wild moose in 1976 (Table 5). The same black fly species were collected from the penned moose in 1976, with the exception of

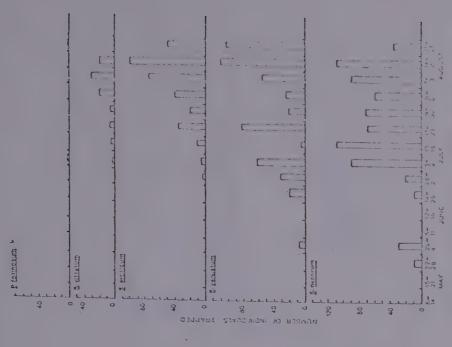
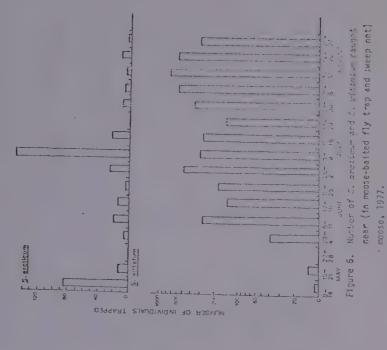
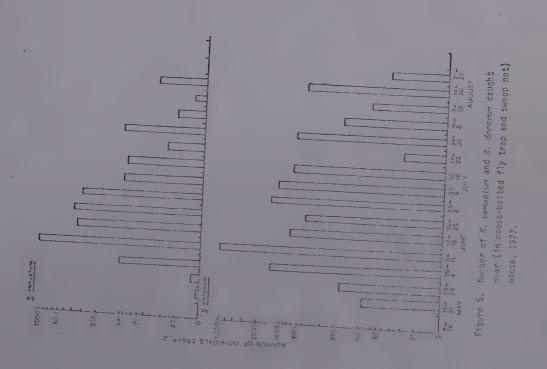


Figure 4. Number of several species of slack files fluct









Simulium jermingsi which was collected in the sweep net on July 15. This is the first known record of S. janningsi in Alberta and identification is based on one female.

Of the 15 species of black files attracted to moost, only

S. arcticism, S. auroum, S. ducorum, S. vornatum, S. vittatism, and
Provination formanian fed on moose. Simultium substicism, J. decorum, and
S. vernatum fed on the penaed moose in both 1976 and 1977, while
S. vittatism and P. formonum, though collected both seasons from the
penned moose (Table 5), only fed on the yearling moose in 1977

(Table 6). Simultium auroum was collected from moose only in 1977.

Table 6: Black filtes engorging on a penned moose, 1976 and 1977.

Species	Number Trapped	Number Engorged	Freedonged
1976	engere disciplicamente per per pasa vidar may publicação por partir de mode, para constituir per	eringiales estatoroporoporoporotestaronominatestaronominatestaronominatestaronominatestaronominatestaronominat	
S. arcticum	323	gava	0.3
S. decorum	648	m	0.5
S. venuetum	355	20	5,0
S. vittatum	53	0	0.0
1977			
S. anoticion	5,628	50	0.7
S. aureun	327	673	0.9
S, decorion	14,879	127	0.0
S. venuation	3,434	231	25
S. vittation	533	36	6.7
P. formcoun	23	الله و	(1) (1) (2)

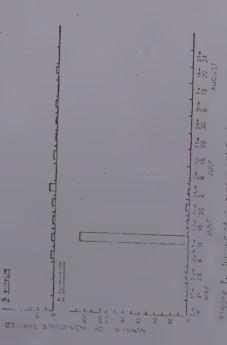


Figure 7. Aurust of 0. Aureum and P. formouum caught near (in moose-balted fly trap and sweep net) moose,



## Determination of Vectors

Of the six species of black files that fed on the penned moose, No microfilariae were found in black files from wild, trapped moose, only S. venustum and S. decorum contained microfilariae (Table 7).

Table 7. Presence of Onchocerca cervipedia microfilariae in blood meals of black flies from a penned moose.

Engenced	No. Examined	No. with	% with
	Philippine and their an energy printing and an energy printing and an energy special particular special part		
0. 127232222	169	U	
S. decoram	101	٦ ،	m
S. arcticum	33	v c	2
S. vittacum	22	<b>&gt;</b> <	0
S. durenn	2	> 0	0
P. formosum	47	D C	0 (
		,	0

erthread with transmission of we causal agents of disease. Briefly, arthropod species and occurrence of infection in a host; 3) repeated Survece (1969) outlined four haste enttenda incriminating an unere must be: 1) effective contact with the bost under matural conditions, 2) a temporal and spatial association of suspected

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demonstration that the specific arthropod harbairs the intectious against controlled conditions. The last bus criteria need further stall burses i definitive statement can be made regarding transmission of leyworm in In the infective stage under natural conditions, and 4] or mastrague moose. In this study, six species of black fly fed on moose and two. satisfying the above criteria. All may be suftable vectors provided transmission of the infectious agent to the definitive asst under Similism decorns and Similism venuetum, ingester large that test they bite the lower legs, and the microfilariae are ingested and complete development in them,

altracted to the penned moose. It and S. verwors on have a wide seographic large maximals (Fredeen 1958; Davies et al. 1962; 252-1977) Species were abundant in mid-June and July when ricretillarian ware recovered from moose. Other flies that fed on miose, S. vitsatur. Simulsian decorms was the rest abundant species of 1, ack fly S. arsticum, S. aurum and Prendmillian formum, whee either most abundant before or after this period or did less feeding on moose distribution (Fredeen 1973) (as does O. corrollywd'n) and Joth (see Table 6).

Unfortunately, no infective stage of G. comigatives found. Microfilariae were found only in S. dwearum and S. venuester.

attracted to the penned moose, being must active in June and 1117. Adults consistently fed on wild, trapped and permed most in the Sunution Desimble Was the second-most abundent species



and 1977. In 1976, 5.6% and in 1977, 6.1% of the females taken in the "Nose-baited fly trap were engonged. This percentage was considered reasonable since Davies (1957) indicated 8 to 25% of the Similium ornation. Concentrated on the Inner and outer aspects of the legs, particularly the hind leg. Reduced activity was noted in the belly, brisket, and anal regions. There was no marked tendency to blood-feed on the ear or head region of moose as observed by Smith in Algonquin Park, Ontario (cited in Anderson and Lankester 1974).

from moose. This multivoltine species is widely distributed in Canada (Freezen 1973). Adults were attracted to the moose-baited fly trap, with 0.5% and 0.9% engorged in 1976 and 1977, respectively. Feeding activity of S. decorum was concentrated on the hind legs of immobilized, wild nouse.

Adults of S. anoticum were abundant throughout the summer with peaks in mid-July and August, suggestive of two generations. Restricted to mestern jorns Averica, this spaces is a blood feeder of horses and cattle (Abdellar, 1969), gathering on the sparsely haired portions of the animal to feed (Peterson 1959). Although adults were attracted to and fed on moose, their restricted geographic distribution may reduce their significance as e vector of O. eventpouls.

in 1975, Liberful active of S. Missanum were collected from Wild, trapped moose, but not from the penned moose. At that time, the penned moose was a calf and may not have had all the correct stimuli to induce engorgement. It is likely that the long, dense natal hair prevented

the black files from reaching the skin to obtain a blood meal. In 1977, when the natal hair was gone, 6.7% of the adults maken from the boose-bailted fly trap were enganged (Table 5).

Similarly, P. formwown was attracted to the penned moose in 1975 but no blood-engorged individuals were collected. In 1977, 54% of those from the moose-baited fly trap were engurged (Table 6), but unlike 'S. buttanme, this species was present in low numbers throughout the summer.

of O. ceruipodia and host-age and prevalence characteristics are similar Young moose, the length and density of the natal Pair possibly certified hair of mouse calves has been shed, the peak period for transmission of to those of Samuel et al. (1976). Prevalence is Figh, particularly in Weinmann et al. (1973) suggested that young Columbian black-tailing men ndicating that the moose had been exposed to the infective stark larrae some protection from biting files. Black files grenally blaceford on year of life in northern California. The possibility extils that issue Results of this study regarding prevalence and general location older moose, and most adult womms occur in the lower front legs. This plus the probable long pre-passent partical the sparsely haired areas of the host. By late July, when the rest. fawns probably missed the peak period of transmission in weir first black files (five S. drooman, the S. Demuscum) taken from the period undetected (see Beaudoin et al. 1970). Seven of the engaged adult may explain the low prevalence of O. carrypodia in joing moose. calves enter winter infected with impace in worse to storeer woose (No. 3) in 1977 contained microfilariae in the blood meal legworm could be over.



as a calf in 1976. This mouse spent from September 1976 to May 1977 in Echonton, Alberta at a location where few black flies are found and O. curvipedia does not occur (W.M.S. unpubl.).

O. correspondes, like many members of this genus, are spatially separated Schulz-Key 1975). Schulz-Key (1975) reported a complex of subcutaneous inus: Nathocerea tarvicola adults were located in the abductor tendons Onchocarea tubingarais adults were found in the caudal part of the back Afcrofflarise were found in skin of the fore and hind limbs, but with the misrofflanius concentrating around the sternum and the inner in the definitive host (Eichler and Melson 1971, Weinmann 1973, and Onchounce flowers inhabited the back and flanks of the host while microfilariae localized in the skin of the inner aspect of the hind of the tible-tarsal or radio-campal joints while the microfilariae localized in the skin of the outer parts of the ear and nose; and filarioids in red deer (Cervus elaphus) from Camany. Adults of were more abundant in hind limbs. Thus microfilariae and adult aspect of the hind legs.

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### ACKAGAL EDGENERTS

Gerry Lynch, Research Biologist, Alberta Fish and Wildlife Division and We express sincere thanks to the field issistings A chard Jurse, Peter Enderwick and Allan Schaaf all of whom worked ong hours under F. J. H. Fredeen, Canada Agriculture, provided his taxonomic Canada, kindly identified or confirmed our identifications of these Dr. R. V. Peterson, Biosystematics Receirch Instituto, Agriculture wildlife technicians Cal Bolomer (deceased) and Orise Lajouresse. specimens of legworm from Columbian black-talled descard and mitthe Special thanks are due to key and Dr. C. J. Weinmann, University of Col fornia, provided less than ideal working conditions. files.



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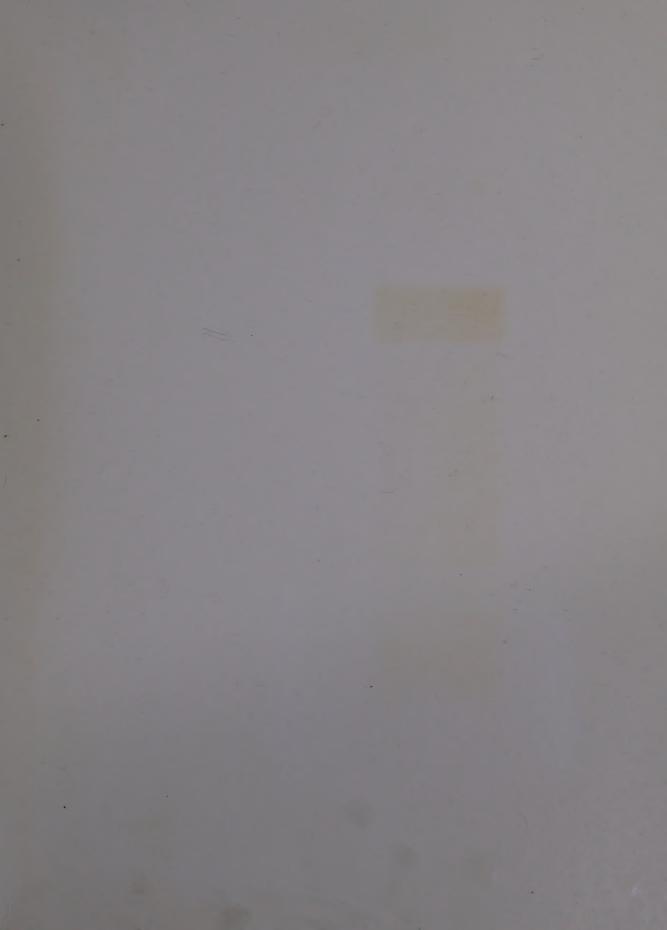
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